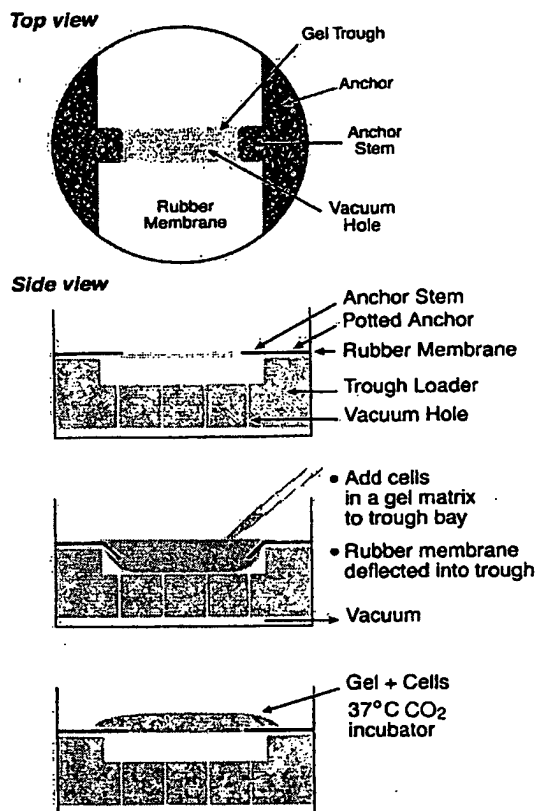


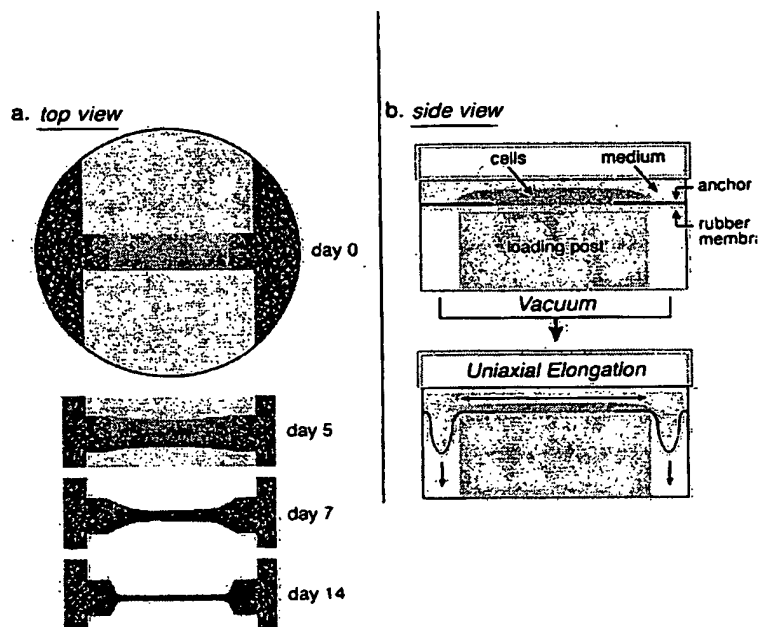
**FIG. 1.** (a) A Delrin Trough Loader insert that is 35 mm in diameter and completely fills the space beneath a well of a Tissue Train culture plate. The trough is  $25 \times 3 \times 3$  mm. The four holes are 1 mm in diameter and communicate with the reservoir beneath the culture plate so that vacuum can draw the overlying rubber membrane into the trough, creating a space into which cells and gel can be cast. Once the gel is cast, the Trough Loader is removed. To mechanically load the bioartificial tissue (BAT), an Arctangle loading post (b) is placed beneath the Tissue Train well so that the linear sides correspond to the east and west poles of the anchors to which the linear gel is attached. Vacuum draws the flexible but inelastic anchors downward, resulting in uniaxial strain on the BAT. (c) A Tissue Train culture plate with linear anchors in each well and two wells with a Trough Loader and Arctangle loading post.

Best Available Copy

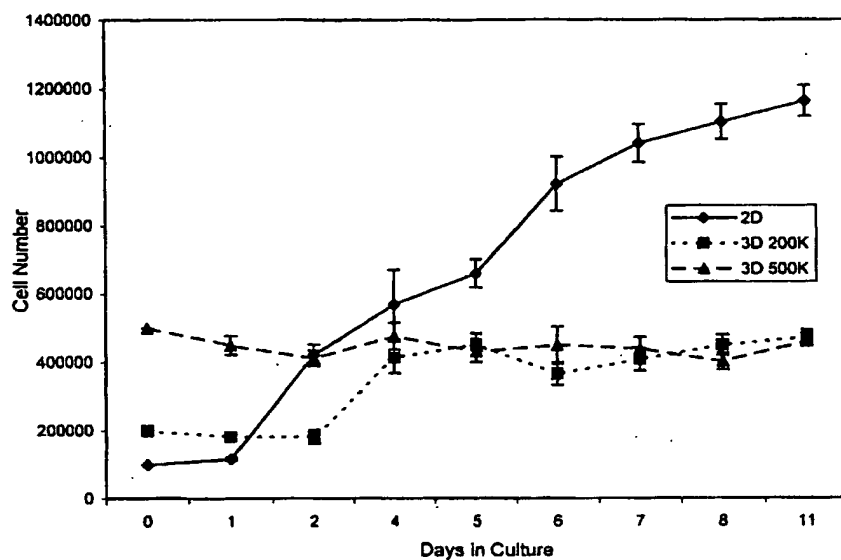


**FIG. 2.** Schematic diagram of one well of a Tissue Train six-well culture plate (top view) shown from above, the gel trough into which the rubber membrane is drawn by vacuum, the non-woven nylon mesh anchor bonded to the rubber in the sector portion, and the anchor stem with collagen bonded to it. Side view: The anchor stem is shown free of the rubber bottom connected to the potted nylon anchor. Vacuum drawn through the Trough Loader holes pulls the rubber membrane downward to closely conform to the trough bay dimensions. Cells in a collagen gel are then added to the trough bay and the constructs are gelled at 37°C in a CO<sub>2</sub> incubator. After gelation, vacuum is released and the cultures receive culture medium.

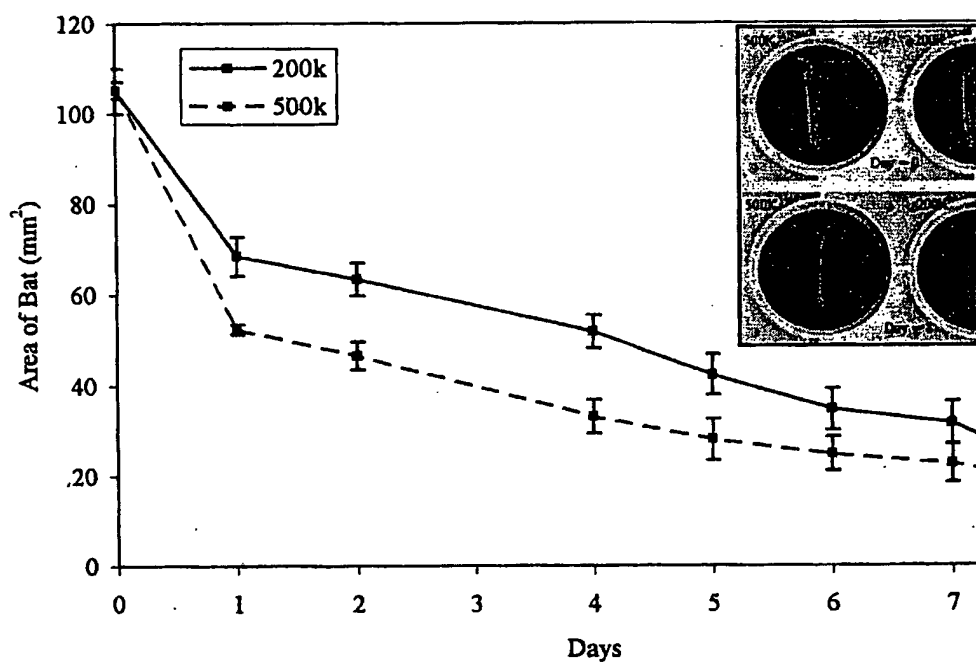
Best Available Copy



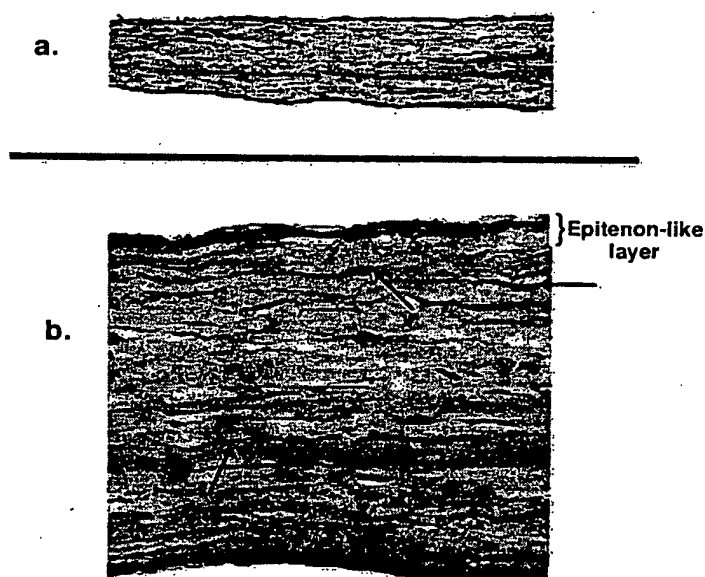
**FIG. 3.** (a) Top view: Dimensions of a typical bioartificial tendon (BAT) from the initial molding on days 0, 5, 7, and 14. The BAT assumes an hourglass shape (days 5 and 7) and finally a cylindrical shape (day 14). (b) Side view: One well of a Tissue Train culture plate with a molded linear BAT immersed in culture medium. The BAT is anchored to the well walls by a rubber membrane. The loading post (rectangle with curved short ends) is positioned below the BAT. When the well bottom the rubber membrane deforms downward at the east and west poles, resulting in uniaxial elongation of the BAT.



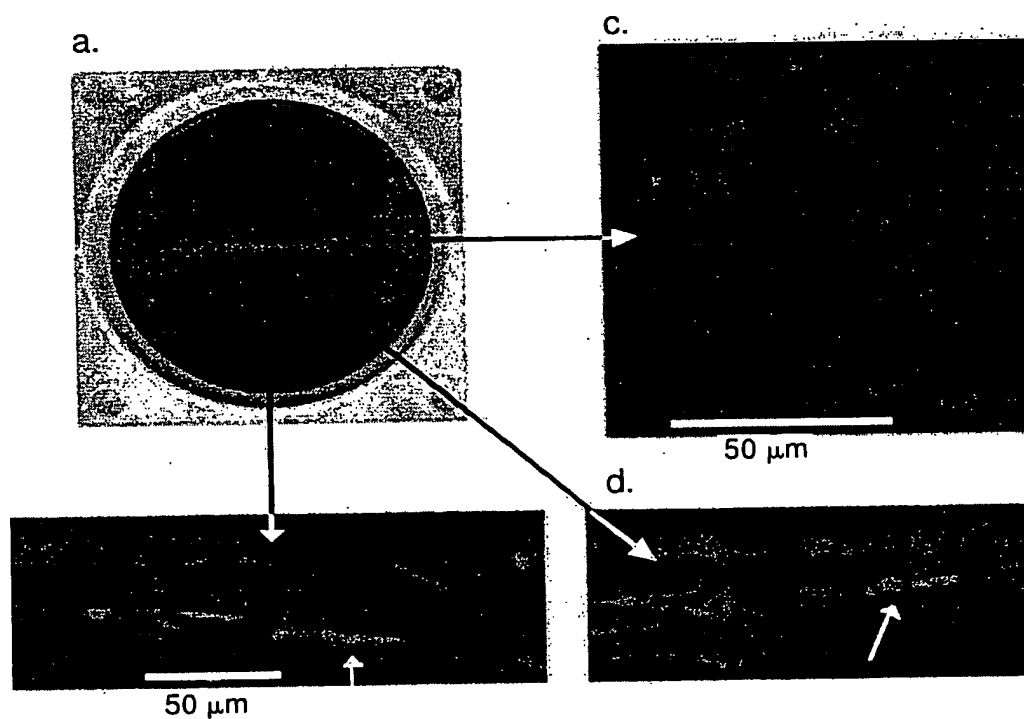
4. Growth curves for avian internal fibroblasts grown in 2-D polystyrene culture dishes covalently bound to collagen and BATs plated at 200K or 500K cells in collagen gels in Tissue Train culture plates. Cells in 2-D dishes grew and passed through several division cycles whereas cells in 3-D gels plated at 200K cells per gel did not divide.



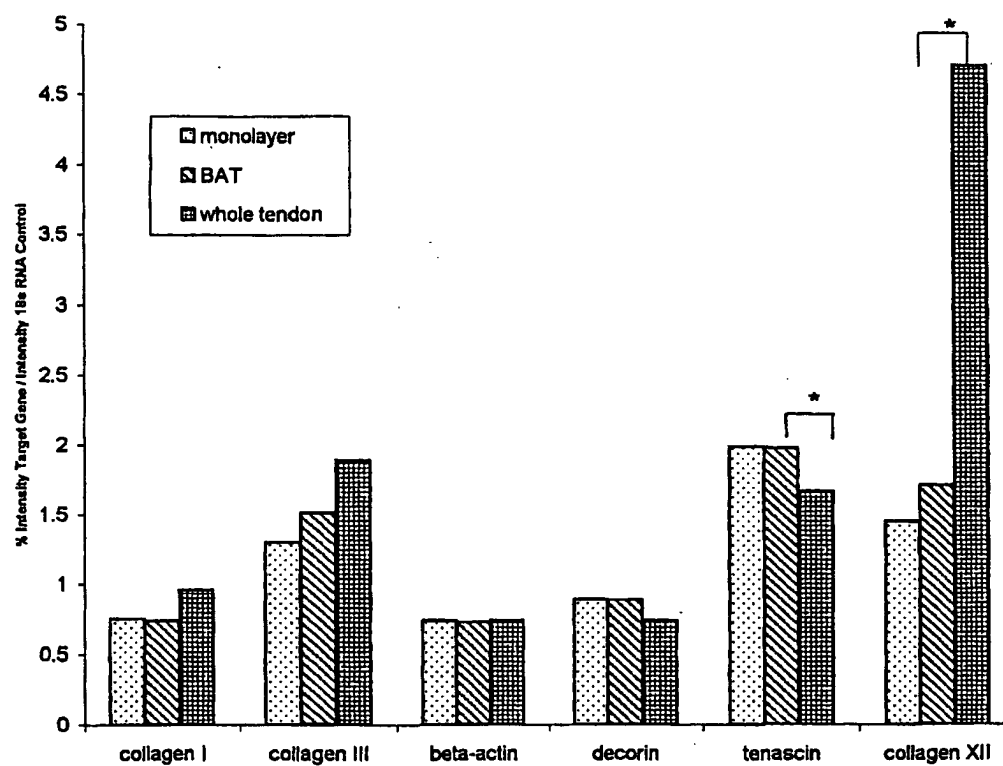
3. 5. Dimensional analyses of bioartificial tendons (BATs) fabricated from 200K or 500K avian tendon BAT. A higher ratio of cells to gel matrix increased the contraction rate.



**FIG. 6.** (a) Longitudinal cross-section of a bioartificial tendon cultured for 10 days in a Tissue Train culturing system, fixed, sectioned, and stained with hematoxylin and eosin (H&E). (b) A higher magnification shows an epitenon-like layer that is two or three cells thick as well as longitudinally aligned tenocytes with elongate basophilic elongate nuclei and cells. Original magnification: (a)  $\times 10$ ; (b)  $\times 40$ .

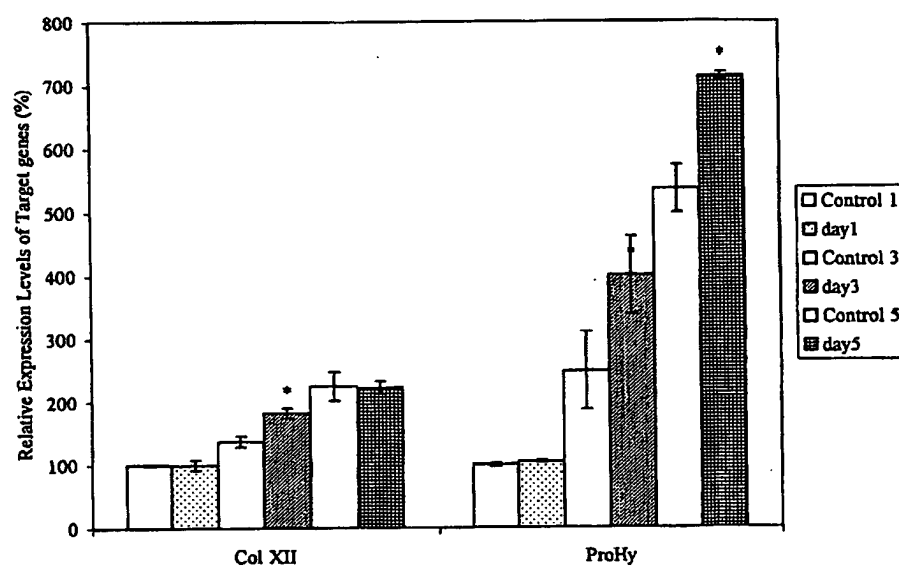


7. (a) A BAT in one well of a Tissue Train culture plate well with a tendon cell-populated collagen construct is contracted and cells are aligned with the principal strain direction except at the stress-shielded region pointing toward 3:00 indicates cells in the collagen gel at the anchor stem region that are stress shielded with the principal strain axis (c). The asterisk in (c) is atop the DAPI-stained nucleus of a tenocyte. (b) and 4:00 (d) shows cells within the collagen gel matrix at the anchor stem-gel matrix border. These cells align along axis of the gel construct and in the principal strain direction. The arrow pointing toward 6:00 (b) section of the BAT that are aligned with the principal strain direction. Nuclei are stained with DAPI and the cells are stained with rhodamine-phalloidin.



**FIG. 8.** Gene expression levels for collagen I, collagen III, collagen XII, decorin, tenascin, and  $\beta$ -actin as markers that are highly expressed in tendon cells. Expression levels were similar for cells grown in 2-D cultures on collagen-bonded surfaces in BATs in collagen gels or in whole tendon. Cells in native tendon expressed slightly less tenascin and about 2.2-fold more collagen XII than 2-D and 3-D counterparts ( $p < 0.05$ ).





**FIG. 9.** Cells in BATs that were mechanically loaded at 1 Hz, 1% elongation for 1 h/day for up to 5 days increased expression levels of collagen XII (Col XII) on day 3 (15%,  $p = 0.05$ ). Prolyl hydroxylase (ProHy) expression was increased 32% on day 3 and more than 2-fold on day 5 in loaded cultures ( $p < 0.05$ ).

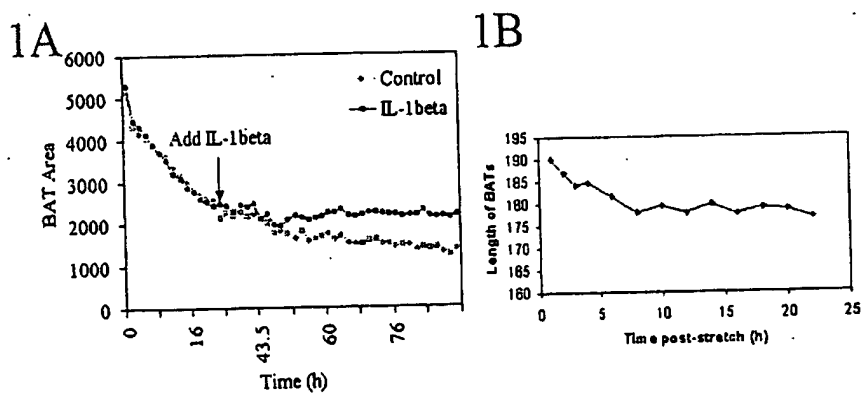


Figure 10. (A). Contraction curves of BATs in the absence or presence of 100 pM IL-1 $\beta$ . (B). Recovery of elongated BATs after maximum stretch.

Best Available Copy

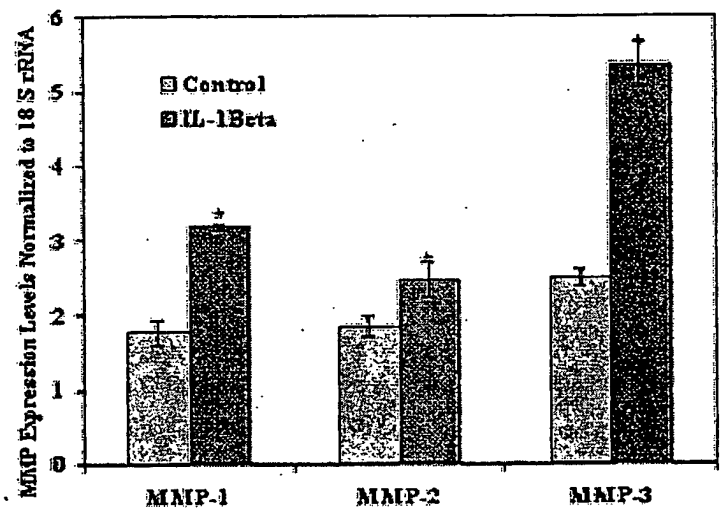


Figure 11. Up-regulation of MMPs by IL-1β.

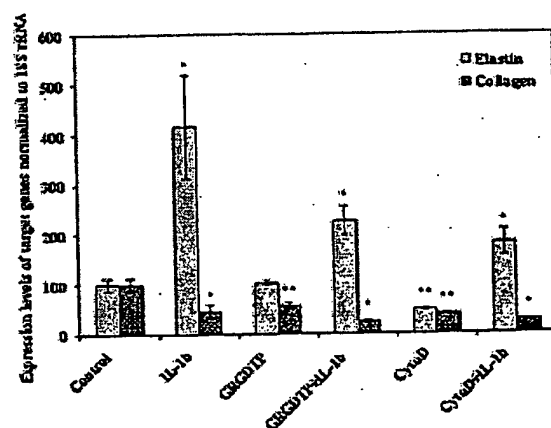


Figure 12. Gene expression of elastin and collagen regulated by IL-1 $\beta$  +/- 10  $\mu$ M cytochalasin D (CytoD) or 100  $\mu$ g/ml GRGDTP. An unpaired Student's t test  $P < 0.01$ . \* indicates the difference between - and + IL-1 $\beta$ , \*\* indicates the difference between - and + cytochalasin D or GRGDTP.

Best Available Copy

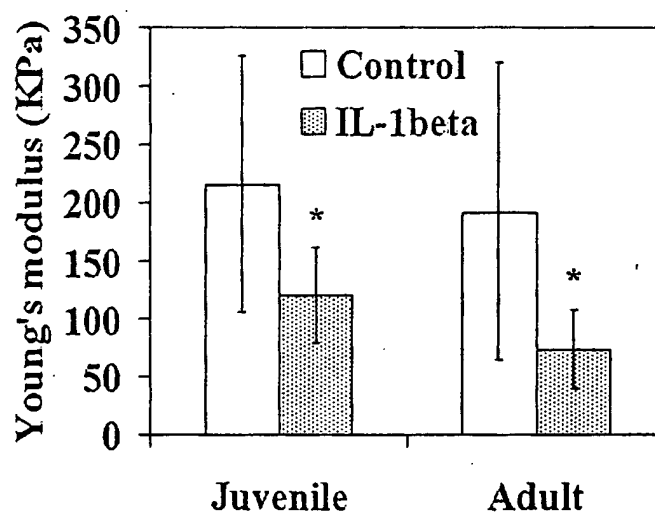


Figure 13. IL-1 $\beta$  reduced cell modulus of monolayer HTIFs from young and adult patients. Unpaired Student's t test,  $P < 0.05$ . Fifteen cells from each group.

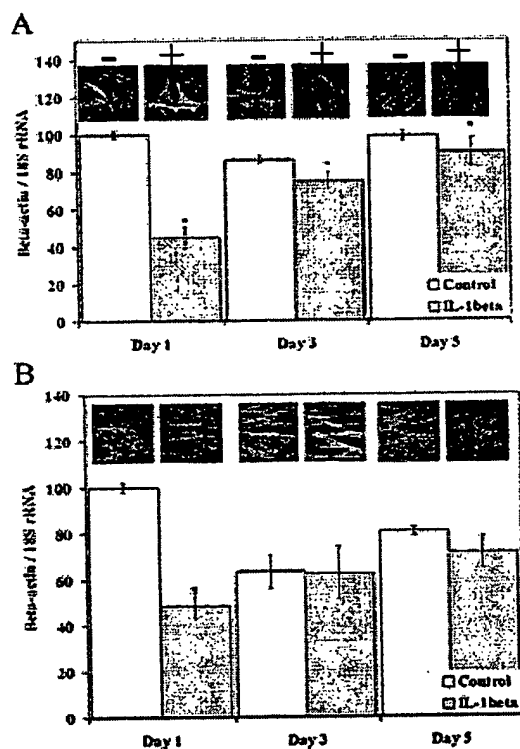


Figure 14. IL-1 $\beta$  down regulated the expression of  $\beta$ -actin. A, in 2D cultures, IL-1  $\beta$  reduced the expression of  $\beta$ -actin at days 1 and 3. At day 5, the protein level of  $\beta$ -actin almost recovered. B, in 3D cultures, the message level of  $\beta$ -actin returned at day 3, but the recover of proteins was delay. Unpaired Student's t test,  $P < 0.05$ .

Best Available Copy

TABLE 1. PCR CONDITIONS USED FOR EACH GENE

<i>Gene</i>	<i>Primer sequence</i>	<i>Product length (bp)</i>	<i>Cycle conditions</i>	<i>Cycles</i>
Collagen I	5'-GGTCCTCAGGGTCTTCTTGG-3' 3'-CACCAGGAGCACCGTTGACT-5'	184	94°C, 5 min 94°C, 1 min; 45°C, 1 min; 72°C, 30 s 72°C, 5 min	28
Collagen III	5'-AGGTGAACGTGGTCCACAAGGT-3' 3'-GCACCAGCTGGTCCAGTCTCT-5'	300	94°C, 5 min 94°C, 1 min; 65°C, 1 min; 72°C, 5 min 72°C, 5 min	26
Collagen XII	5'-AGTATCAGTCTGGGCCTGGCAA-3' 3'-TTTCTCCCTCTCCAGAAAGGGCTT-5'	300	94°C, 5 min 94°C, 1 min; 65°C, 1 min; 72°C, 1 min 72°C, 5 min	35
Decorin	5'-CATCCCTTACTGAGCTTCACCTT-3' 3'-ACTCACACCAGAATAGGTTGCCTG-5'	300	94°C, 5 min 94°C, 1 min; 65°C, 1 min; 72°C, 1 min 72°C, 5 min	22
Tenascin	5'-TGTCTACAACATCAAGCTGCCTGT-3' 3'-AGCCTGCCTTACCTTCTGCTGT-5'	298	94°C, 5 min 94°C, 1 min; 65°C, 1 min; 72°C, 1 min 72°C, 5 min	37
Prolyl-hydroxylase	5'-AACAGCCAATGAAGTAGAGGCAGT-3' 3'-ACGACAATGCGTTGGGTACTCA-5'	300	94°C, 5 min 94°C, 1 min; 60°C, 1 min; 72°C, 1 min 72°C, 5 min	22
$\beta$ -Actin	5'-GCCATCCTGCGTCTGGACCGGGCT-3' 3'-GTGATGACCTGGCCGTCAGGCAGC-5'	227	94°C, 5 min 94°C, 1 min; 60°C, 1 min; 72°C, 30 s 72°C, 5 min	20

TABLE 2. COMPARISON OF MODULUS OF ELASTICITY AND ULTIMATE TENSILE STRENGTH  
RESULTS FOR MECHANICALLY CONDITIONED AND CONTROL SPECIMENS ON DAY 7

	<i>Load</i>	<i>No load</i>
Day 7 elasticity (MPa)	$1.80 \pm 1.82$	$0.49 \pm 0.24$
Day 7 ultimate tensile strength (kPa)	$327.65 \pm 172.03$	$112.20 \pm 6.07$